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In Vitro and In Vivo Antifungal Activities of BMY-28864, a Water-Soluble Pradimicin Derivative

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BMY-28864, a water-soluble pradimicin derivative, had potent in vitro activity against a wide variety of fungi, including those associated with deep-seated mycosis; it inhibited the growth of standard strains and clinical isolates at concentrations of 12.5 μg/ml or less. At the MIC or higher concentrations, BMY-28864 was fungicidal for *Candida albicans* under both growing and nongrowing conditions. BMY-28864 expressed fungicidal activity only in the presence of Ca^{2+}, and its activity was totally diminished when ethylene glycol-bis(2-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), a Ca^{2+} chelator, was added to the test medium. The effectiveness of intravenously administered BMY-28864 in vivo was examined and compared with that of amphotericin B in mouse models of fungal infections. Both normal and cyclophosphamide-treated immunosuppressed mice infected with *C. albicans*, *Cryptococcus neoformans*, or *Aspergillus fumigatus* responded to therapy with BMY-28864 (50% protective doses of 17, 18, and 37 mg/kg of body weight in normal mice and of 32, 35, and 51 mg/kg in cyclophosphamide-treated mice, respectively). Lethal lung infections were also established with *C. albicans* or *A. fumigatus* in cyclophosphamide-treated mice. The 50% protective doses of BMY-28864 were 15 and 23 mg/kg per dose against *C. albicans* and *A. fumigatus*, respectively. The immunosuppression induced by intraperitoneal administration of 200 mg of cyclophosphamide per kg lasted for 5 days, and total recovery was observed by day 7.

The rising incidence of systemic fungal infections in patients with suppressed immune systems brought about by the use of cytotoxic drugs, immunosuppressive therapy, or human immunodeficiency virus infection and the prolonged treatments that are necessary in these patients have highlighted the shortcomings of existing antifungal therapies. Amphotericin B remains the drug of choice for life-threatening fungal infections despite the relatively high degree of toxicity associated with its use, often a limiting factor in practice. Therefore, a need exists for fungicidal, broad-spectrum, less-toxic antifungal agents to supplement the list of currently available antifungal drugs.

Pradimicin A is the original member of the pradimicins produced by *Actinomadura hibisca* P157-2 (ATCC 53557) (14, 15, 23, 24). It possesses potent activity in vitro against a wide range of pathogenic fungi (16) and demonstrates in vivo efficacy against a large number of *Candida albicans* isolates (3). Although pradimicin A is relatively nontoxic, its limited solubility in aqueous media at physiological pHs posed difficulties in further development. As part of the program aimed at identifying water-soluble derivatives, we embarked on microbial and chemical modification studies of pradimicin A (6, 7, 18, 20) and developed BMY-28864 (13) (Fig. 1).

BMY-28864 was found to have broad and potent antifungal activity comparable to that of pradimicin A in vitro. It was water soluble (>40 mg/ml at pH 7.2) and well tolerated in mice; no acute lethal or apparent side effects were noted during 10 days of observation following an intravenous administration of up to 600 mg of BMY-28864 per kg of body weight. These properties would allow it to be readily formulated for intravenous administration. BMY-28864 was therefore examined in several fungal-infection models in mice with normal immune and suppressed immune functions. In this report, we present the data on in vitro antifungal activity of BMY-28864 under a variety of conditions and compare the in vivo efficacy of BMY-28864 with that of amphotericin B against *C. albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* infections in normal and cyclophosphamide (CY)-treated immunosuppressed mice.

MATERIALS AND METHODS

Antibiotics. BMY-28864 was prepared at Bristol-Myers Squibb Research Institute, Tokyo, Japan. A 10-mg/ml stock solution of the drug was prepared by dissolving BMY-28864 in 10% dimethyl sulfoxide (DMSO) and adjusting the pH of the solution to 7.4 with 1 M sodium hydroxide. Twofold dilutions of this solution were made with 10% DMSO. Amphotericin B was formulated by dissolving Fungizone (amphotericin B-sodium deoxycholate complex; product of Bristol-Myers Squibb K.K.) in 10% DMSO, providing a stock solution of 2 mg/ml. Twofold dilutions were made with water. Ketoconazole obtained from Janssen Pharmaceuticals was suspended in 10% DMSO and solubilized by adding small amounts of 1 M hydrochloric acid, providing a stock solution of 1 mg/ml. Twofold dilutions were made with 10% DMSO.

Media and buffers. Yeast nitrogen base, neopeptone, and yeast morphology agar (YMA) were purchased from Difco Laboratories, Detroit, Mich. Sheep whole blood was purchased from Nippon Bio-supplies Center, Tokyo, Japan. Fetal bovine serum was purchased from Biocell Laboratories, Carson, Calif. Yeast nitrogen broth containing 1% glucose (YNBG) was prepared as specified by the manufacturer, adjusted to pH 7.0 with 1 M sodium hydroxide, and filter sterilized (cellulose nitrate membrane; pore size, 0.2 μm). YNBG buffered with 0.1 M phosphate, pH 7.0, was

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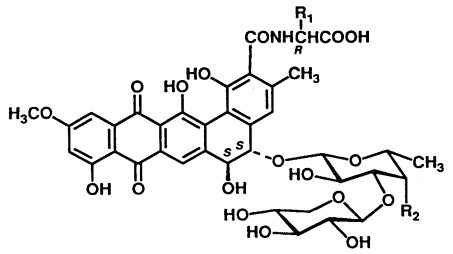

FIG. 1. Chemical structures of BMY-28864 and pradimicin A. In BMY-28864, R_1 is CH_2OH and R_2 is $N(CH_3)_2$. In pradimicin A, R_1 is CH_3 and R_2 is $NHCH_3$. *R* and *S*, *R* and *S* configurations, respectively.

prepared similarly. Sabouraud dextrose broth (SDB) was prepared with 1% neopeptone and 4% glucose and filter sterilized. A 100 mM solution of ethylene glycol-bis(2-aminoethyl ether)-*N*,*N*,*N*',*N*'-tetraacetic acid (EGTA; Sigma), pH 7.2, was prepared by adding sodium hydroxide to a suspension of EGTA in distilled water and filter sterilized. As yeast nitrogen broth contains about 1 mM Ca^{2+}, YNBG medium containing 2 mM EGTA was used for the experiment in which the Ca^{2+} requirement was investigated. YMA buffered with phosphate was used to study the effect of medium pH. YMA containing 0.1 M KH_2PO_4 was adjusted to an appropriate pH with 5 M sodium hydroxide and autoclaved (121°C, 15 min). Dulbecco's phosphate-buffered saline containing 0.9 mM Ca^{2+} and 0.5 mM Mg^{2+} [PBS(+)], pH 7.2, was prepared with phosphate-buffered salt (Flow Laboratories; catalog no. 28-103-05), $CaCl_2$, and $MgCl_2$ that had been autoclaved separately.

Fungi. Forty-two fungal isolates were used for in vitro studies. They consisted of 4 strains of *Saccharomyces cerevisiae*, 10 strains of *C. albicans* (3 standard strains and 7 locally isolated clinical isolates), 15 strains of *Candida tropicalis* (1 standard strain and 14 clinical isolates), 2 strains of *Candida parapsilosis*, 2 strains of *Candida guilliermondii*, 2 strains of *Candida krusei*, 2 strains of *Candida glabrata*, 2 strains of *Candida kefyr*, and 1 strain each of *C. neoformans*, *A. fumigatus*, and *Trichophyton mentagrophytes*. Clinical isolates of *C. albicans* (83-2-14, 83-8-29, and 85-12-17) were kindly provided by H. Ikemoto, Juntendo University, Tokyo, Japan. Clinical isolates of *C. tropicalis* (85-8, 85-46, 85-593, 86-1-4, and 86-236) were kindly provided by H. Kume, Kitasato University, Tokyo, Japan. Clinical isolates of *C. tropicalis* (MTU 12034, MTU 12036, MTU 12052, MTU 12058, MTU 12059, and MTU 12099) were kindly provided by K. Nishimura, Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba, Japan. Clinical isolates of *C. albicans* (M 1001, M 1012, M 1445, and M 1447), *C. tropicalis* (M 1017, M 1519, and M 1525), *C. guilliermondii* (M 1002 and M 1023), *C. krusei* (M 1005 and M 1006), *C. glabrata* (M 1008 and M 4002), and *C. kefyr* (M 1004 and M 1007) were kindly provided by T. Shinoda, Meiji College of Pharmacy, Tokyo, Japan. *C. albicans* A9540, *C. neoformans* IAM 4514, and *A. fumigatus* IAM 2034 were used for both in vitro and in vivo experiments. Isolates were stored at −80°C in 0.067 M phosphate buffer (pH 7.2) containing 20% glycerol. Yeasts were grown at 28°C in yeast extract-peptone-glucose broth (YPG; yeast extract, 0.4%; polypeptone, 1.0%; glucose, 1.5%; K_2HPO_4, 0.05%; $MgSO_4$, 0.05% [pH 7.0]) for 18 h with shaking. A 1-ml suspension was inoculated into fresh YPG (100 ml) and grown at 28°C for 5 h with shaking. Both *A. fumigatus* IAM 2034 and *T. mentagrophytes* 4329 were grown at 28°C on YPG–1.5% agar slants for 14 days, and spore suspensions were prepared by washing spores from the agar surface with 0.067 M phosphate buffer containing 0.2% (vol/vol) Tween 80. Inocula in each case were adjusted by hemacytometer counting.

Susceptibility testing. Broth dilution MICs were determined in 250-μl volumes in 96-well microtiter plates (Sumitomo Bakelite Co., Tokyo, Japan). Nine parts of yeast suspension (or spore suspension of *A. fumigatus* IAM 2034) were mixed with one part of antibiotic dilution. Inocula were adjusted to 10^5 cells per ml, except in experiments in which the effect of inoculum sizes was investigated. The plates were incubated at 37°C for 24 h without agitation, and the turbidity of individual wells was measured at 620 nm. MICs were defined as the lowest antibiotic concentrations that prevented visible growth, and 75% inhibitory concentrations were calculated from the turbidity readings of drug-treated cultures relative to those of untreated cultures that reached the stationary phase. The MIC results reported are those generated in at least two separate runs which agreed within a twofold range.

Agar dilution MICs were determined on phosphate-buffered YMA. Nine parts of molten agar were combined with one part of antibiotic dilution in petri dishes. A 5-μl suspension containing 2×10^6 cells per ml (2×10^7 cells per ml for *T. mentagrophytes* 4329) was spotted on the surface of the agar plates. The plates were incubated at 28°C for 60 h. MICs were recorded after 40 h of incubation except for the *T. mentagrophytes* isolate, which required 60 h to produce visually evaluable growth on the drug-free control plates. Agar dilution MICs were defined as the lowest antibiotic concentrations showing no growth or less than five discrete colonies per spot.

Determination of fungicidal activity. Cells of *C. albicans* A9540 grown at 28°C for 5 h in YPG were collected by centrifugation. The effect of BMY-28864 on growing cells was studied by incubating cells (initial inoculum, 2×10^5 CFU/ml) in YNBG, pH 7.0, containing a fourfold series of antibiotic concentrations. Incubation of 20-ml volumes was at 28°C for 48 h in 100-ml Erlenmeyer flasks with shaking (100 rpm). Drug-free control cells grew only in the blastoconidial phase without forming any germ tubes or pseudohyphae under these conditions. Samples (1-ml aliquots) were removed at 1, 2, 4, 8, 24, and 48 h, and the antibiotic was inactivated by diluting it 10- to 1,000 fold with 0.067 M phosphate buffer containing 2 mM EGTA (pH 7.2). The number of viable cells was determined from duplicate plate counts made on YPG–1.5% agar. The minimal accurately detectable number of viable cells was 5×10 CFU/ml.

The effect of BMY-28864 on nongrowing cells was studied by incubating cells (initial inoculum, 4×10^6 CFU/ml) in Dulbecco's PBS(+), pH 7.2, containing a fourfold series of antibiotic concentrations. Incubation of 20-ml volumes was at 28°C for 24 h in 100-ml Erlenmeyer flasks with shaking (100 rpm). Samples (1-ml aliquots) were removed at 1, 2, 4, 8, and 24 h, and the number of viable cells was determined by plating as described above for the experiment with growing cells.

Experimental infections in mice. Experimental systemic and lung infections were studied in specific-pathogen-free male ICR mice, each weighing 20 to 24 g. Neutropenia was induced by intraperitoneal administration of 200 mg of CY (Shionogi, Osaka, Japan) per kg 4 days before the fungal infection. This is a minor modification of the procedures described by others (1, 2, 5, 12) and produced a 70%

TABLE 1. Broth dilution MICs of BMY-28864

Organism[a] (no. of isolates)	MIC (μg/ml)[b] for individual isolates
S. cerevisiae (4)	1.6, 3.1 (3)
C. albicans (10)	3.1 (2), 6.3 (8)
C. tropicalis (15)	3.1 (2), 6.3 (12), 12.5
C. parapsilosis (2)	6.3 (2)
C. guilliermondii (2)	3.1, 6.3
C. krusei (2)	3.1 (2)
C. glabrata (2)	1.6 (2)
C. kefyr (2)	1.6 (2)
C. neoformans (1)	1.6
A. fumigatus (1)	3.1

[a] Inoculum size, 10^5 cells per ml.
[b] Determined by the broth dilution method in YNBG, pH 7.0. Numbers in parentheses are numbers of isolates with the indicated MIC.

reduction in the peripheral blood leukocyte count on the day of infection, resulting in an approximately fivefold increase in susceptibility to systemic fungal infections compared with that of normal mice. The suppression lasted for 5 days after the dose of CY, and total recovery was observed by day 7. For systemic infections, groups of five normal or neutropenic mice at each dose level were intravenously infected with *C. albicans* A9540 (1.0×10^6 cells per normal mouse or 2.2×10^5 cells per neutropenic mouse, i.e., 10 times the 50% lethal dose [LD_{50}]), *C. neoformans* IAM 4514 (1.0×10^6 cells per normal mouse or 2.9×10^5 cells per neutropenic mouse [10 LD_{50}]), or *A. fumigatus* IAM 2034 (1.0×10^7 cells per normal mouse or 2.0×10^6 cells per neutropenic mouse [10 LD_{50}]) via the lateral tail vein. Test compounds were intravenously administered once immediately after the infection. For lung infections, the method of Shibuya et al. (22) was adopted. Groups of 10 neutropenic mice at each dose level were anesthetized intraperitoneally with 35 mg of sodium pentobarbital (Somnopentyl; Pitman-Moore, Inc., Washington Crossing, N.J.) per kg and infected with *C. albicans* A9540 (1.0×10^6 cells per mouse [10 LD_{50}]) or *A. fumigatus* IAM 2034 (1.1×10^8 cells per mouse [10 LD_{50}]) intranasally. Test compounds were intravenously administered three times at 48-h intervals beginning immediately after the infection. The 50% protective dose (PD_{50}) was calculated by the method of Litchfield and Wilcoxon (8) from the survival rate recorded 20 days after the challenge. Untreated animals died 2 to 11 days postinfection.

RESULTS

Antifungal activity of BMY-28864. Susceptibilities of 41 strains (10 standard strains and 31 locally isolated clinical isolates) to BMY-28864 were determined in YNBG by the broth dilution method. As summarized in Table 1, all strains including those associated with deep-seated mycosis were susceptible to BMY-28864 (MIC ranges in 41 strains, 1.6 to 12.5 μg/ml). Estimates of relative potency against strains of *C. albicans* and *C. tropicalis* in vitro suggest that amphotericin B may be 16- to 32-fold more potent than BMY-28864 on a microgram-per-milliliter basis. On the other hand, amphotericin B was at least 300-fold more hemolytic than BMY-28864; amphotericin B at 3.1 μg/ml was lytic to sheep erythrocytes when incubated at 37°C in PBS(+) for 1 h, while BMY-28864 at 1,000 μg/ml had no lytic effect on erythrocytes under the same conditions.

Effect of inoculum size. When the starting inoculum was varied from 10^4 to 10^6 cells per ml for the two strains of *C. albicans*, the MICs of BMY-28864 increased (Table 2). This inoculum size effect was, however, much smaller than that observed for ketoconazole.

TABLE 2. Effect of inoculum size on broth dilution MICs of BMY-28864, amphotericin B, and ketoconazole

C. albicans strain	Inoculum size (cells/ml)	MIC (μg/ml)[a] of: BMY-28864 YNBG	BMY-28864 SDB	Amphotericin B YNBG	Amphotericin B SDB	Ketoconazole in YNBG
A9540	10^4	3.1	12.5	0.4	0.4	6.3 (0.0016)
	10^5	6.3	12.5	0.4	0.4	50 (0.0031)
	10^6	12.5	25	0.4	0.4	100
ATCC 32354	10^4	3.1	6.3	0.4	0.4	3.1 (0.0031)
	10^5	6.3	6.3	0.4	0.4	25 (0.0063)
	10^6	12.5	12.5	0.4	0.4	50

[a] Determined by the broth dilution method after 24 h of incubation at 37°C in YNBG buffered with 0.1 M phosphate (pH 7.0) or SDB (pH 7.0). Numbers in parentheses denote concentrations that inhibited fungal growth by 75%.

Effects of medium pH and serum. No variations in MICs of BMY-28864 were noted as the medium pH varied from 5.0 to 8.5 in phosphate-buffered YMA (Table 3). Similarly, addition of fetal bovine serum at concentrations of up to 50% did not affect the activity of BMY-28864 (Table 4). The activity of ketoconazole, on the other hand, was dependent on both the medium pH and the concentration of serum.

Fungicidal activity. BMY-28864 was fungicidal to cells of *C. albicans* A9540 under both growing and nongrowing conditions. With an inoculum of 2×10^5 CFU/ml in YNBG, BMY-28864 at the MIC (6.3 μg/ml) or higher concentrations yielded a 3-log reduction in viable cells in 8 h or less (Fig. 2). With an inoculum of 4×10^6 CFU/ml in PBS(+), BMY-28864 at 6.3 μg/ml affected a 2-log reduction in viable cells in 24 h (Fig. 3).

Requirement of Ca^{2+} for antifungal activity of BMY-28864. When five strains of *C. albicans* (A9540, ATCC 32354, 83-2-14, 83-8-29, and 85-12-17), six strains of *C. tropicalis* (IFO 10241, 85-8, 85-46, 85-593, 86-236, and 86-1-4), and one

TABLE 3. Effect of medium pH on agar dilution MICs of BMY-28864 and ketoconazole

Organism[a]	MIC (μg/ml)[b] of: BMY-28864 pH 5.0	BMY-28864 pH 7.0	BMY-28864 pH 8.5	Ketoconazole pH 5.0	Ketoconazole pH 7.0	Ketoconazole pH 8.5
S. cerevisiae ATCC 9763	1.6	1.6	NG	3.1	3.1	NG
C. albicans A9540	3.1	3.1	3.1	100	25	3.1
C. albicans ATCC 32354	3.1	3.1	3.1	100	25	3.1
C. tropicalis 85-8	6.3	6.3	3.1	100	50	12.5
C. tropicalis IFO 10241	6.3	6.3	3.1	50	25	0.8
C. neoformans IAM 4514	1.6	0.8	0.8	3.1	0.8	0.4
A. fumigatus IAM 2034	3.1	3.1	3.1	50	6.3	6.3
T. mentagrophytes 4329	1.6	3.1	3.1	3.1	1.6	0.2

[a] Inoculum size, 10^4 cells per spot (10^5 cells per spot for *T. mentagrophytes* 4329).
[b] Determined by the agar dilution method after 60 h of incubation at 28°C on YMA containing 0.1 M KH_2PO_4 that had been adjusted to the appropriate pHs with 1 M NaOH. NG, no growth after 60 h of incubation.

TABLE 4. Effect of serum on broth dilution MICs of BMY-28864 and ketoconazole

Organism[a]	MIC (μg/ml)[b] of:					
	BMY-28864			Ketoconazole		
	0%[c]	20%	50%	0%	20%	50%
C. albicans A9540	6.3	6.3	6.3	25 (0.05)	50 (12.5)	100 (50)
C. albicans ATCC 32354	3.1	3.1	6.3	50 (0.05)	100 (50)	100
C. tropicalis 85-8	6.3	6.3	12.5	12.5 (6.3)	50 (25)	100
C. neoformans IAM 4514	3.1	3.1	3.1	50	>100	>100

[a] Inoculum size, 10^5 cells per ml.
[b] Determined by the broth dilution method after 24 h of incubation at 37°C in YNBG that had been supplemented with 0, 20, or 50% fetal bovine serum. Numbers in parentheses denote concentrations that inhibited fungal growth by 75%.
[c] Concentration of serum.

strain each of *S. cerevisiae* (ATCC 9763), *C. neoformans* (IAM 4514), and *A. fumigatus* (IAM 2034) were tested in YNBG containing 2 mM EGTA, BMY-28864 at 100 μg/ml was found to be ineffective in preventing organism growth. EGTA at concentrations of up to 2 mM did not affect the growth of these strains or alter their susceptibilities to amphotericin B (MICs ranged from 0.2 to 0.4 μg/ml). To further study the effect of Ca^{2+} on the activity of BMY-28864, the following experiment was carried out. Exposure of *C. albicans* A9540 (4×10^6 CFU/ml) to 25 μg of BMY-28864 per ml for 2 h at 28°C in PBS(+) resulted in a nearly 1-log reduction in viable cells, but the addition of EGTA at a final concentration of 2 mM and further incubation at 28°C for 22 h did not affect viability (Fig. 3). As much as 7 μg of BMY-28864 per 4×10^6 CFU bound to *C. albicans* cells in PBS(+) but was recovered quantitatively when the antibiotic-bound cells were washed with 2 mM EGTA.

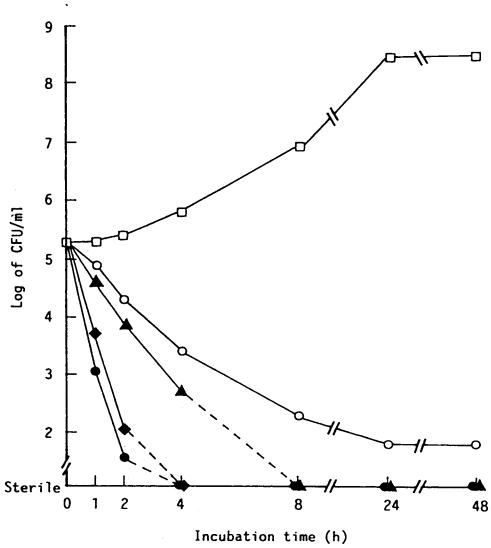

FIG. 2. Effect of BMY-28864 (6.3 μg/ml, ○; 25 μg/ml, ▲; 100 μg/ml, ◆) and amphotericin B (1.6 μg/ml, ●) on the number of viable cells of *C. albicans* A9540 at 28°C in YNBG, pH 7.0. □, drug-free control.

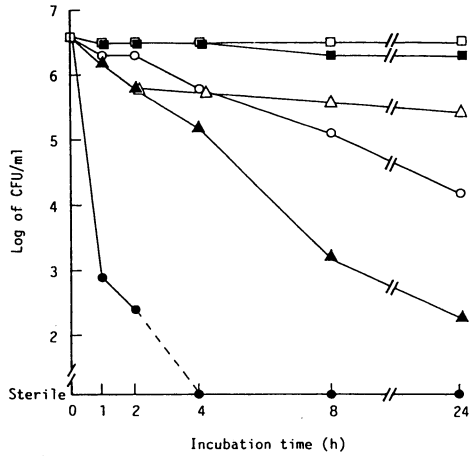

FIG. 3. Effect of BMY-28864 (1.6 μg/ml, ■; 6.3 μg/ml, ○; 25 μg/ml, ▲) and amphotericin B (1.6 μg/ml, ●) on the number of viable cells of *C. albicans* A9540 at 28°C in PBS(+), pH 7.2. △, EGTA at 2 mM added after 2 h of exposure to 25 μg of BMY-28864 per ml; □, drug-free control.

Effect of BMY-28864 or amphotericin B on mouse survival during systemic infections. BMY-28864 and amphotericin B were comparatively evaluated in lethal systemic infections in normal and CY-treated immunosuppressed mice by a single intravenous administration. The results are summarized in Table 5. By day 20, all untreated control mice died. BMY-28864 therapy at 25 or 50 mg/kg significantly prolonged the survival of *C. albicans* A9540- or *C. neoformans* IAM 4514-infected mice. The PD_{50}s were 17 and 18 mg/kg in normal mice and 32 and 35 mg/kg in CY-treated mice, respectively. Against systemic aspergillosis, BMY-28864 therapy at 50 mg/kg resulted in the survival of 60 and 40% of normal and CY-treated mice, respectively. BMY-28864 ther-

TABLE 5. Comparative in vivo efficacies of BMY-28864 and amphotericin B for treatment of experimental systemic candidiasis, cryptococcosis, and aspergillosis in normal and CY-treated mice[a]

Organism[b]	Drug[c]	PD_{50}[d] (mg/kg) for mice	
		Normal	CY treated
C. albicans A9540	BMY-28864	17 (11–26)	32 (21–50)
	Amphotericin B	0.35 (0.23–0.54)	0.51 (0.39–0.67)
C. neoformans IAM 4514	BMY-28864	18 (13–24)	35 (26–48)
	Amphotericin B	0.46 (0.25–0.87)	0.51 (0.39–0.67)
A. fumigatus IAM 2034	BMY-28864	37 (24–57)	51 (39–67)
	Amphotericin B	0.46 (0.25–0.87)	0.38 (0.25–0.58)

[a] Male ICR mice (20 to 24 g) were intraperitoneally injected with 200 mg of CY per kg 4 days prior to fungal infection.
[b] Inoculum size, 10 LD_{50} of each organism in both normal and CY-treated mice.
[c] Intravenously injected one time immediately after fungal infection.
[d] Determined 20 days after fungal infection. Values in parentheses are 95% confidence limits.

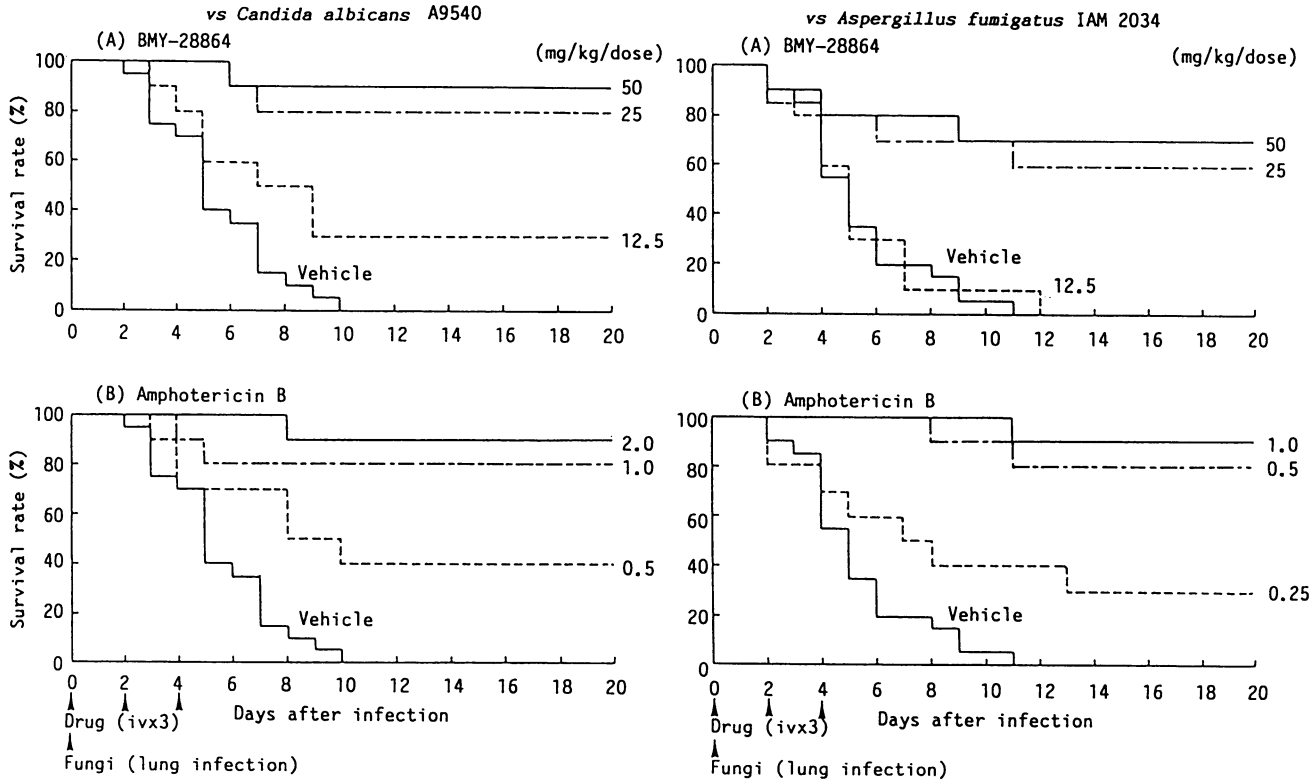

FIG. 4. Antifungal activities of BMY-28864 and amphotericin B against *C. albicans* A9540 and *A. fumigatus* IAM 2034 lung infections in CY-treated mice. Male ICR mice (20 to 24 g) were intraperitoneally injected with 200 mg of CY per kg 4 days prior to fungal infection with a 10-LD_{50} inoculum of each organism (1.0×10^6 cells per mouse for *C. albicans* A9540 or 1.1×10^8 cells per mouse for *A. fumigatus* IAM 2034). Drugs were intravenously (iv) injected three times at 48-h intervals beginning immediately after fungal infection.

apy at 100 mg/kg resulted in the survival of 100% of infected mice in all these infection models. Amphotericin B therapy at 1.0 or 2.0 mg/kg significantly prolonged the survival of infected mice and is thus clearly superior to BMY-28864 therapy in regard to animal survival. Whereas amphotericin B at 4.0 mg/kg could not be assayed because of lethality, mice receiving BMY-28864 at 600 mg/kg appeared to be well throughout the 10-day observation period.

Effect of BMY-28864 or amphotericin B on mouse survival during lung infections. BMY-28864 and amphotericin B were comparatively evaluated in lethal lung infections in CY-treated immunosuppressed mice. Test compounds were intravenously administered three times at 48-h intervals beginning immediately after infection. The results are summarized in Fig. 4. BMY-28864 therapy at 25 mg/kg per dose on days 0, 2, and 4 prolonged the survival of 80 and 60% of *C. albicans* A9540- and *A. fumigatus* IAM 2034-infected mice, with PD_{50}s of 15 and 23 mg/kg per dose, respectively.

DISCUSSION

BMY-28864 showed potent in vitro activity against a wide variety of fungi. Although not as potent as amphotericin B, BMY-28864 was more selective and much safer than amphotericin B. MICs for individual strains showed little variation when determined by the broth dilution method in YNBG. SDB tends to yield slightly higher MICs than YNBG. The MICs for the two strains of *C. albicans* increased when the starting inoculum was raised (Table 2). This increase is much smaller than those reported for azole antifungal agents (4, 9–11, 17). The modest inoculum size effect obtained with BMY-28864 may be due to the high level of binding to yeast cells. With an extremely high inoculum, irreversible binding may result in a situation in which the concentration of the antibiotic in the test medium is not adequate to prevent the growth of all the cells.

BMY-28864 was fungicidal to *C. albicans* under both growing and nongrowing conditions. It should be noted, however, that BMY-28864 expressed fungicidal activity only in the presence of Ca^{2+}. The antifungal activity was totally diminished when EGTA, a Ca^{2+} chelator, was added to the test medium (Fig. 3). As has been postulated for pradimicin A (21) and subsequently for BMY-28864 (19), the mode of action of pradimicin is believed to begin with the strong Ca^{2+}-dependent binding of the antibiotic to the cell wall mannan and mannoprotein. This is likely to result in changes in the three-dimensional arrangements of the cell wall polymers and in the contacts between cell wall and cell membrane, thus affecting, for example, natural cell wall autolysis with insertion of new cell wall materials and thereby causing alterations in membrane integrity. Although the precise mechanism of action of the pradimicin class of antifungal agents is not well understood, the fact that the fungicidal effect and mannan binding of the antibiotic were totally reversed with EGTA suggests that BMY-28864 is effective only when continuously bound to the cell wall components. BMY-28864 proved to be a well-tolerated drug in mice (13) and had no lytic effect on sheep erythrocytes in vitro. Our belief is that the selective toxicity of BMY-28864 to fungal

cells is due to its specific binding to the fungal cell wall mannan and mannoprotein.

The present study demonstrated that the in vitro fungicidal activity of BMY-28864 was clearly translated into in vivo results. BMY-28864 given to normal mice by a single intravenous dosage was effective in systemic *C. albicans*, *C. neoformans*, and *A. fumigatus* infections, with PD_{50} values of 17, 18, and 37 mg/kg, respectively. BMY-28864 given by the same dosing schedule significantly prolonged the survival of CY-treated immunosuppressed mice infected systemically with *C. albicans*, *C. neoformans*, or *A. fumigatus*. Estimates of relative effectiveness and toxicity suggest that amphotericin B may be approximately 50-fold more effective and at least 130-fold more toxic than BMY-28864 on a milligram-per-kilogram level. In another experiment, a remarkable increase in the efficacy of BMY-28864 against systemic candidiasis was seen with a once-daily 5-day intravenous dosing schedule; the PD_{50} was 5.1 mg/kg per dose (data not shown).

Clearly, no experimental animal model completely simulates the complexities of individual neutropenic patients. Nevertheless, the lung models adopted in this study are considered to reflect a major route of lethal fungal infections in humans. Although the results are preliminary and detailed animal studies are needed, the effectiveness against *C. albicans* and *A. fumigatus*, good aqueous solubility [>40 mg/ml in PBS(+), pH 7.2], and low toxicity of BMY-28864 indicate the clinical potential of this compound for the prevention and treatment of opportunistic fungal infections in immunocompromised hosts.

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